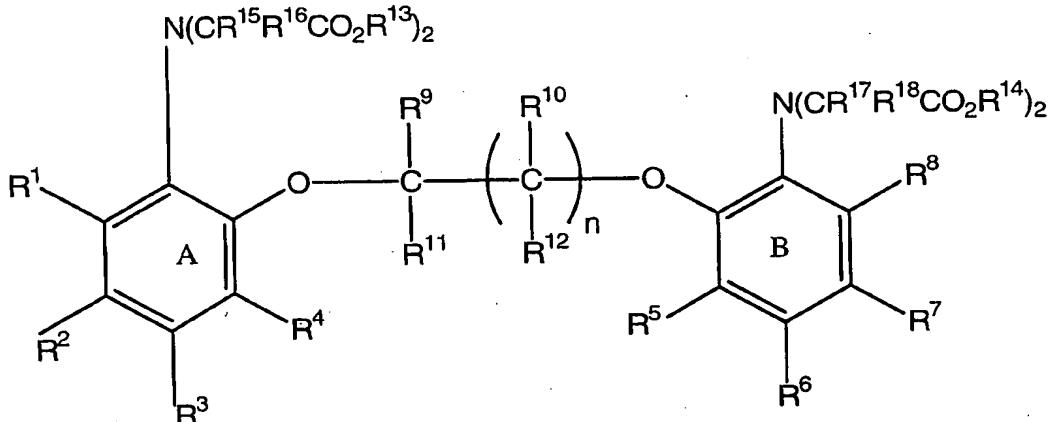


**CLAIMS**

What is claimed is:

- 5 1. A binding solution comprising:
  - a) a BAPTA metal chelating moiety;
  - b) a salt comprising trivalent metal ions; and,
  - c) an acid.
- 10 2. A binding solution comprising:
  - a) a phosphate-binding compound having formula  $(A)m(L)n(B)$  wherein A is a chemical moiety, L is a linker, B is a metal-chelating moiety, m is an integer from 1 to 4 and n is an integer from 0 to 4;
  - b) a salt comprising trivalent metal ions; and,
  - c) an acid.
- 15 3. The binding solution according to Claim 2, wherein said binding solution has a pH about 3 to about pH 6.
- 20 4. The binding solution according to Claim 3, wherein said metal ion is selected from the group consisting of  $Ga^{3+}$ ,  $Fe^{3+}$  and  $Al^{3+}$ .
5. The binding solution according to Claim 4, wherein said salt is gallium chloride.
- 25 6. The binding solution according to Claim 5, wherein said chemical moiety is a label that is a dye, an enzyme or a hapten provided that the dye is not sulfonated or said chemical moiety is a reactive group.
- 30 7. The binding solution according to Claim 6, wherein said hapten is biotin.
8. The binding solution according to Claim 6, wherein said dye is selected from the group consisting of a benzofuran, a quinazolinone, a xanthene, an indole, a benzazole and a boropolyazaindacene.
- 35 9. The binding solution according to Claim 6, wherein said metal-chelating moiety is BAPTA.

10. The binding solution according to Claim 9, wherein said compound has the formula (A) $m$ (L) $n$ (B) wherein A is a chemical moiety that is a dye or a reactive group, L is a linker, m is an integer from 1 to 4, n is an integer from 0 to 4 and B is a metal-chelating moiety having said Formula IV comprising;



#### **FORMULA IV**

10

wherein said dye or reactive group is attached to at least one of R<sup>1</sup>-R<sup>12</sup> by a linker or at least one of R<sup>1</sup>-R<sup>8</sup> in combination with ring A or ring B forms a dye;

15 R<sup>1</sup>-R<sup>8</sup> that are not a dye or reactive group are independently selected from the group consisting of hydrogen, halogen, alkoxy, alkyl, aryl, amino, carboxyl, nitro, cyano, thioether, hydroxyl, sulfinyl and linker;

20 R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup> and R<sup>12</sup>, are independently selected from the group consisting of hydrogen, linker and lower alkyl, or adjacent substituents R<sup>9</sup> and R<sup>10</sup> in combination constitute a 5-membered or 6-membered alicyclic or heterocyclic ring;

$R^{15}$ ,  $R^{16}$ ,  $R^{17}$  and  $R^{18}$  are independently hydrogen, lower alkyl or alkyl, wherein alkyl or lower alkyl is optionally substituted by carboxyl or alkoxy;

25

n is 1 or 2; and,

R<sup>13</sup> and R<sup>14</sup> are independently hydrogen or a salt.

11. The binding solution according to Claim 10, wherein at least one of said R<sup>1</sup>-R<sup>4</sup> is independently a dye or reactive group and R<sup>5</sup>-R<sup>8</sup> is independently selected from the group consisting of H, NO<sub>2</sub>, F, CF<sub>3</sub>, lower alkyl, and linker wherein said linker is optionally attached to a biotin, a reactive group or a dye.  
5
12. The binding solution according to Claim 11, wherein said dye is independently R<sup>3</sup>, R<sup>2</sup> or R<sup>3</sup> and R<sup>2</sup> together.  
10
13. The binding solution according to Claim 11, wherein said R<sup>6</sup> or R<sup>7</sup> is a linker and said linker is optionally attached to a biotin, a reactive group or dye.  
15
14. The binding solution according to Claim 11, wherein said R<sup>6</sup> and R<sup>5</sup> are independently fluorine.  
15
15. The binding solution according to Claim 11, wherein said R<sup>6</sup> is NO<sub>2</sub>.  
20
16. The binding solution according to Claim 10, wherein said phosphate-binding compounds having said formula (A)m(L)n(B) are selected from the group consisting of Compound 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12.  
25
17. The binding solution according to Claim 2, wherein said binding solution further comprises an organic solvent and a buffering agent.  
30
18. The binding solution according to Claim 17, wherein said organic solvent is acetonitrile.  
30
19. The binding solution according to Claim 9 or Claim 10, wherein said phosphate-binding compounds are in solution or immobilized on a solid or semi-solid matrix.  
35
20. A method for binding a phosphorylated target molecule in a sample, said method comprising the steps of:
  - i) contacting said sample with a binding solution according to any one of claims 1-19; and,

- ii) incubating said sample and said binding solution for sufficient time to allow said phosphate-binding compound to associate with said phosphorylated target molecule whereby said phosphorylated target molecule is bound.
- 5      21. The method according to Claim 20, wherein said method further comprises illuminating said phosphate-binding compound with a suitable light source whereby said labeled phosphorylated target molecule is detected.
- 10     22. The method according to Claim 20, wherein said phosphorylated target molecule is selected from the group consisting of proteins, peptides, nucleotides, carbohydrates, phosphatase substrates, kinase substrates, lipids and inorganic phosphate.
- 15     23. The method according to Claim 22, wherein said phosphorylated target molecules are immobilized on a solid or semi-solid matrix or are solubilized in solution.
- 20     24. The method according to Claim 23, wherein said solid or semi-solid matrix is a gel, a membrane, a polymeric particle, or an array.
- 25     25. A method for detecting a phosphorylated target molecule in a sample immobilized on a membrane, said method comprising the steps of:
- i) optionally electrophoretically separating said sample on a gel;
  - ii) immobilizing said sample to a membrane;
  - iii) optionally contacting said membrane of step ii) with a fixing solution;
  - iv) contacting said membrane of step iii) with a binding solution according to any one of Claims 2-19, wherein said binding solution comprises:
    - a) a phosphate-binding compound having formula (A)<sub>m</sub>(L)<sub>n</sub>(B) wherein A is a chemical moiety, L is a linker, B is a metal-chelating moiety, m is an integer from 1 to 4 and n is an integer from 0 to 4;
    - b) a salt comprising metal ions; and,
    - c) an acid;
  - v) incubating said membrane of step iv) and said binding solution for sufficient time to allow said phosphate-binding compound to associate with said phosphorylated target molecule; and,
  - vi) visualizing said compound whereby said phosphorylated target molecule is detected.

26. The method according to Claim 25, wherein step iii) further comprises contacting said membrane with a wash solution following contact with said fixing solution.

27. The method according to Claim 26, wherein said phosphorylated target molecule is  
5 selected from the group consisting of proteins, peptides, and nucleotides.

28. The method according to Claim 27, wherein said phosphate-binding compounds having said formula  $(A)m(L)n(B)$  wherein B comprises Formula VI are selected from the group consisting of Compound 1, 2, 3, 4, 5, 9, 10, 11 and 12.

10 29. The method according to Claim 28, wherein said step v) further comprises contacting said membrane with a wash solution following contact with said binding solution.

15 30. The method according to Claim 29, further comprising adding an additional detection reagent to said membrane.

31. The method according to Claim 30, wherein said additional detection reagent is a staining solution specific for total proteins, a staining solution specific for glycoproteins or an antibody.

20 32. A method for detecting a phosphorylated target molecule in a sample immobilized on a gel, said method comprising the steps of:

- i) immobilizing said sample on a gel;
- 25 ii) optionally contacting said gel of step i) with a fixing solution;
- iii) contacting said gel of step ii) with a binding solution according to any one of claims 2-19, wherein said binding solution comprises:
  - a) a compound having formula  $(A)m(L)n(B)$  wherein A is a chemical moiety, L is a linker, B is a metal-chelating moiety, m is an integer from 1 to 4 and n is an integer from 0 to 4;
  - b) a salt comprising metal ions; and,
  - c) an acid;
- 30 iv) incubating said gel of step iii) and said binding solution for sufficient time to allow said compound to associate with said phosphorylated target molecule; and,
- 35 v) visualizing said compound whereby said phosphorylated target molecule is detected.

33. The method according to Claim 32, wherein said phosphorylated target molecules are selected from the group consisting of proteins, peptides, and nucleotides.
- 5 34. The method according to Claim 33, wherein said phosphate-binding compounds having said formula  $(A)m(L)n(B)$  wherein B comprises Formula VI are selected from the group consisting of Compound 1, 2, 3, 4, 5, 9, 10, 11 and 12.
- 10 35. The method according to Claim 34, further comprising adding an additional detection reagent to said gel.
36. The method according to Claim 35, wherein said additional detection reagent is a staining solution specific for total proteins or a staining solution specific for glycoproteins.
- 15 37. The method according to Claim 34, wherein step ii) further comprises contacting said gel with a wash solution following contact with said fixing solution.
- 20 38. The method according to Claim 34, wherein said method further comprises first electrofocusing said sample before step i.
39. The method according to Claim 34, wherein said step i of said method further comprise electrophoretically separating said sample.
- 25 40. The method according to Claim 34, wherein said step v) further comprises contacting said gel with a wash solution following contact with said binding solution.
41. A method for detecting a phosphorylated target molecule in a sample immobilized on a matrix, said method comprising the steps of:
- 30 i) immobilizing said sample on an matrix;
- ii) contacting said matrix of step i) with a binding solution according to any one of Claims 2-19, wherein said binding solution comprises;
- 35 a) a phosphate-binding compound having formula  $(A)m(L)n(B)$  wherein A is a chemical moiety, L is a linker, B is a metal-chelating moiety, m is an integer from 1 to 4 and n is an integer from 0 to 4;
- b) a salt comprising metal ions; and,
- c) an acid;

- iii) incubating said matrix of step ii) and said binding solution for sufficient time to allow said compound to associate with said phosphorylated target molecule; and,
- iv) visualizing said compound whereby said phosphorylated target molecule is detected.

5 42. The method according to Claim 41, wherein said sample is selected from the group consisting of proteins, peptides, nucleotides, lipids, kinase substrates, phosphatase substrates, phosphate binding proteins and carbohydrates.

10 43. The method according to Claim 42, wherein said matrix is a polymeric gel, glass, polymeric membrane, plastic, polymeric microparticle.

15 44. The method according to Claim 43, wherein said phosphate-binding compound having said formula  $(A)m(L)n(B)$  wherein B comprises Formula VI is selected from the group consisting of Compound 1, 2, 3, 4, 5, 9, 10, 11 and 12.

20 45. The method according to Claim 44, wherein said step iii) further comprises contacting said matrix with a wash solution following contact with said binding solution.

46. The method according to Claim 45, further comprising adding an additional detection reagent to said matrix.

25 47. The method according to Claim 45, wherein said additional detection reagent is a staining solution specific for total proteins, a staining solution specific for glycoproteins or an antibody.

48. A method for isolating phosphorylated target molecules from a sample, said method comprising the steps of:

- 30 i) contacting said sample with a binding solution according to any one claims 1-19; and,
- ii) incubating said sample of step i) and said binding solution for sufficient time to allow said compound to associate with said phosphorylated target molecule to form a ternary complex; and,
- 35 iii) separating said complex from said sample whereby said phosphorylated target molecules are isolated.

49. The method according to Claim 48, wherein said binding solution further comprises acetonitrile.
- 5 50. The method according to Claim 49, wherein said separating further comprises precipitating said complex.
- 10 51. The method according to Claim 50, wherein said separating further comprises addition of a base solution to said precipitated complex wherein said complex is dissociated whereby said phosphorylated target molecules are isolated by affinity chromatography.
- 15 52. The method according to Claim 50 or Claim 51, wherein said separating optionally further comprises addition of an organic extraction solution to said complex of Claim 50 or Claim 51 whereby said phosphorylated target molecules are isolated from said phosphate-binding compound.
- 20 53. The method according to Claim 52, wherein said phosphorylated target molecules are selected from the group consisting of proteins, peptides and nucleotides.
- 25 54. The method according to Claim 53, wherein said compound having said formula  $(A)m(L)n(B)$  wherein B is Formula IV are selected from the group consisting of compound 1, 2, 3, 4, 5, 6, 9, 10, 12, 15, and 16.
55. A method for isolating phosphorylated target molecules from a sample, said method comprising the steps of:
- 30 i) charging a matrix comprising a metal-chelating moiety selected from the group consisting of Formula IV with a salt comprising trivalent metal ions;
- ii) equilibrating said matrix with an acidic binding buffer,
- iii) adding said sample to said matrix wherein said phosphorylated target molecules are bound to said matrix of step ii);
- iv) eluting said phosphorylated target molecules from said matrix with a base solution whereby said phosphorylated target molecules are isolated.
- 35 56. The method according to Claim 55, wherein said matrix is selected from the group consisting of polymeric particles, polymeric membrane, glass and plastic.

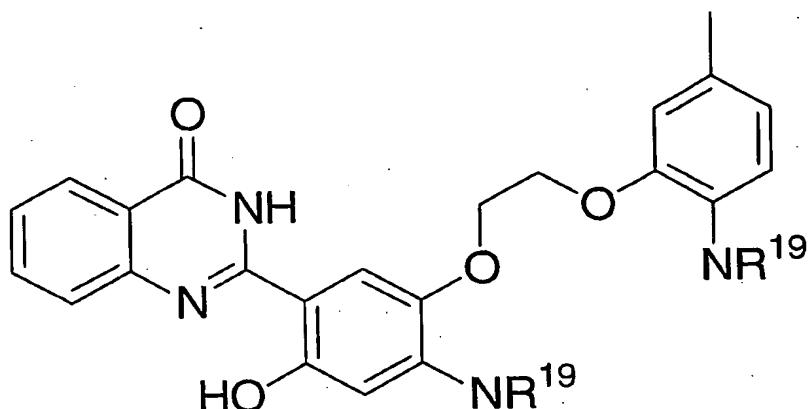
57. The method according to Claim 56, wherein said phosphorylated target molecules  
are proteins, peptide or nucleotides.
- 5 58. The method according to Claim 57, wherein said acidic binding buffer has a pH about  
3 to about 6.
- 10 59. The method according to Claim 58, wherein said trivalent metal ions are selected  
from the group consisting of Ga<sup>3+</sup>, Fe<sup>3+</sup> and Al<sup>3+</sup>.
- 15 60. The method according to Claim 59, wherein said salt is gallium chloride.
61. The method according to Claim 59, wherein said matrix comprises Compound 2, 5,  
13, 14, 15 and 16.
- 15 62. A kit for binding a phosphorylated target molecule in a sample, said kit comprising:  
  
i) a binding solution according to any one of Claims 1-19; and,  
ii) wherein said kit optionally includes molecular weight markers that comprise  
20 phosphorylated and non-phosphorylated polypeptides.
63. The kit according to Claim 62, wherein said kit independently further comprises a  
fixing solution, additional detection reagent, standards or a wash solution.
- 25 64. The kit according to Claim 63, wherein said kit further comprises a matrix
65. The kit according to Claim 64, wherein said matrix further comprises a phosphatase  
or kinase substrate.
- 30 66. The kit according to Claim 63, wherein said additional detection reagent is  
independently a staining solution specific for total proteins, a staining solution specific  
for glycoproteins or antibodies.
- 35 67. The kit according to Claim 62, wherein said phosphate-binding compounds having  
said formula (A)m(L)n(B) are selected from the group consisting of Compound 1, 2,  
3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15 and 16.

68. A matrix comprising an immobilized phosphate-binding compound according to Formula (A)<sub>m</sub>(L)<sub>n</sub>(B) wherein A is a chemical moiety, L is a linker, B is a metal chelating moiety according to Formula IV, m is an integer from 1 to 4 and n is an integer from 0 to 4.

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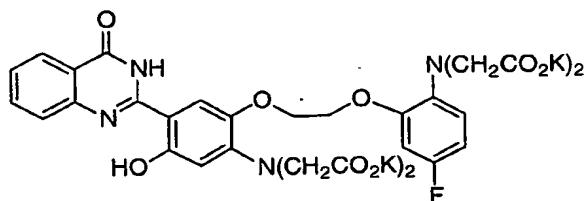
69. A compound having formula (A)<sub>m</sub>(L)<sub>n</sub>(B) wherein A is a label, L is a linker, m is an integer from 1 to 4, n is an integer from 0 to 4 and B is a metal-chelating moiety comprising Formula IV wherein said compounds are selected from the group consisting of

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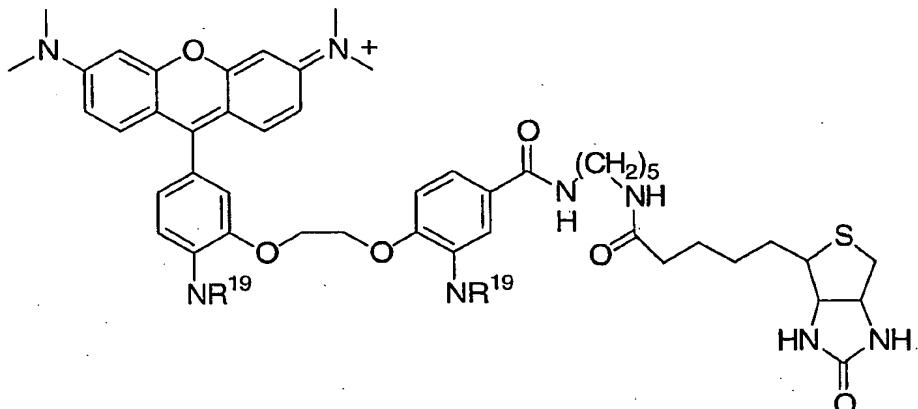


Compound 6,

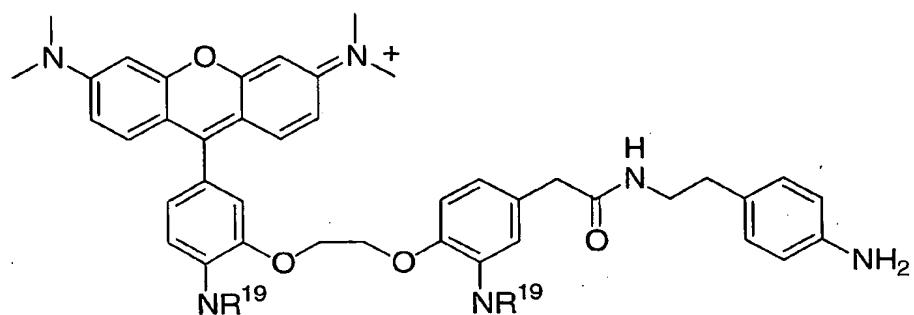
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Compound 7,

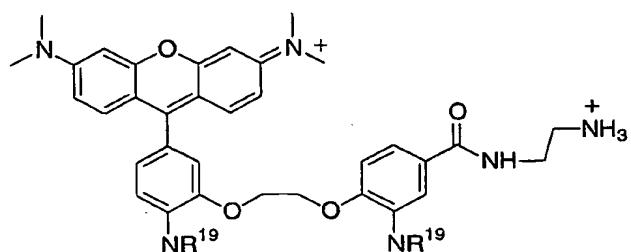


Compound 9,



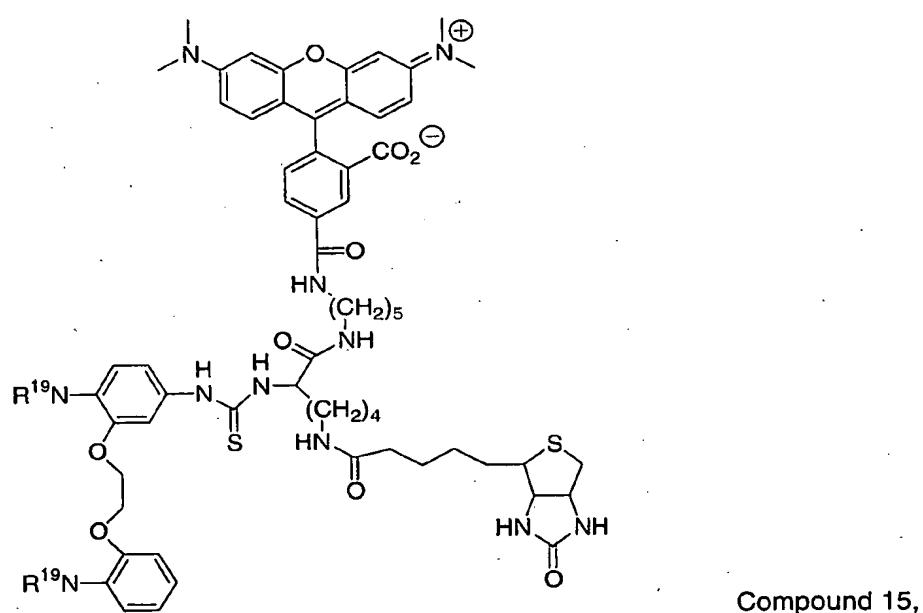
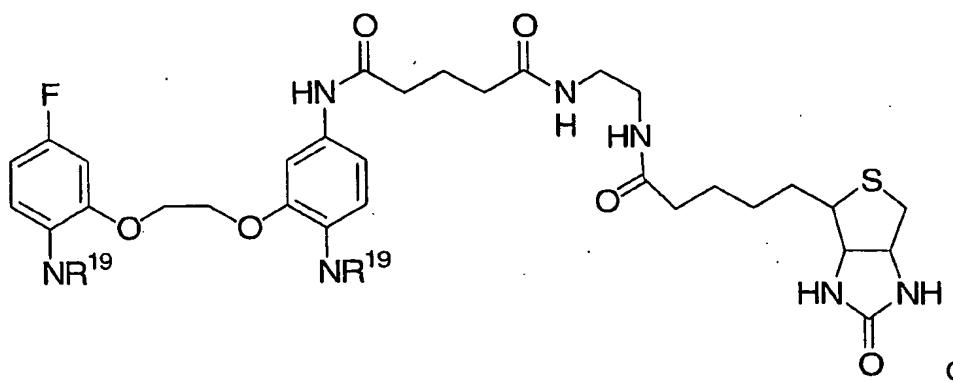
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Compound 10,

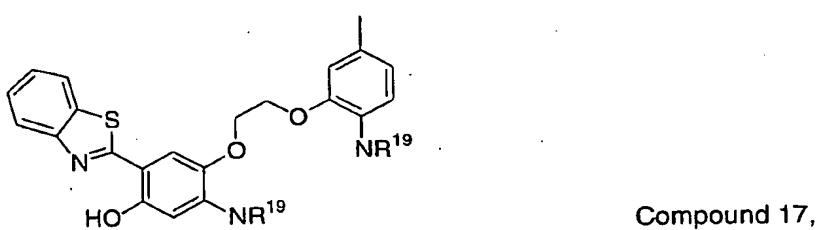


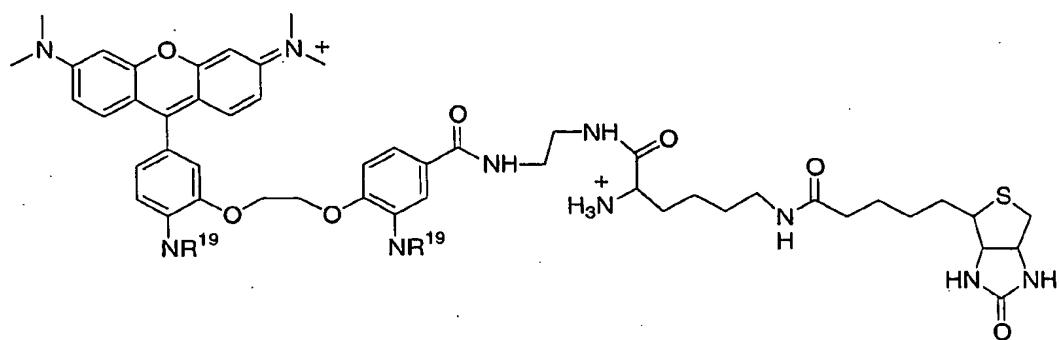
Compound 11,

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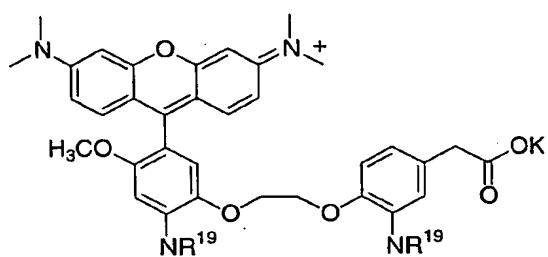


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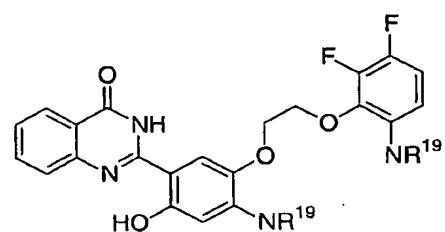


Compound 18,



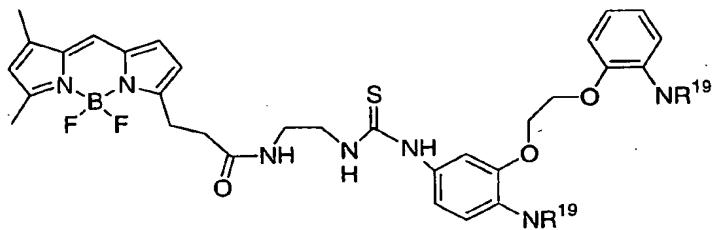
Compound 19,

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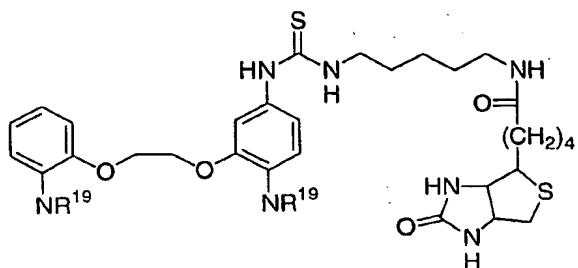


Compound 23,

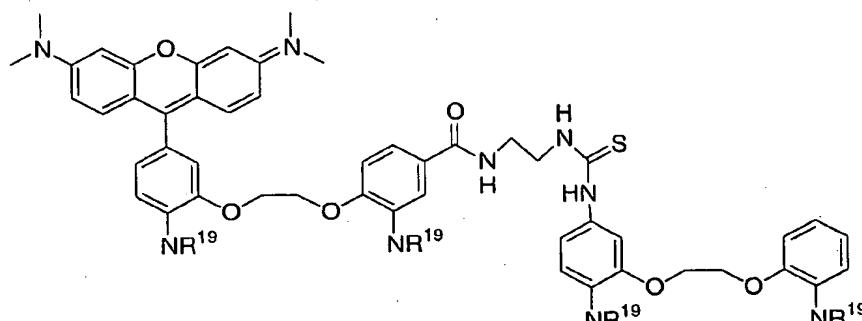
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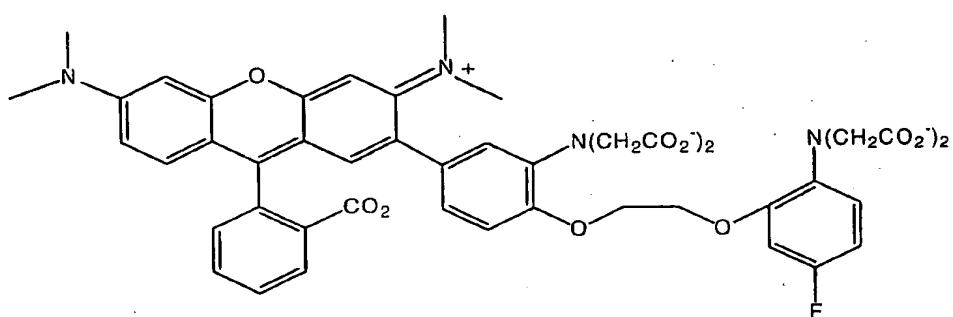
Compound 24,



## Compound 25



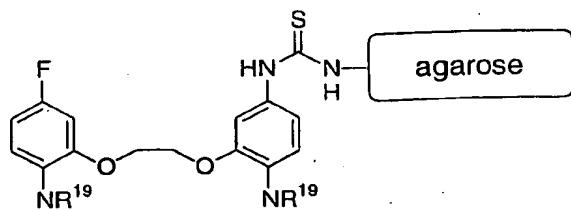
NR<sup>19</sup> NR<sup>19</sup> Compound 26 and



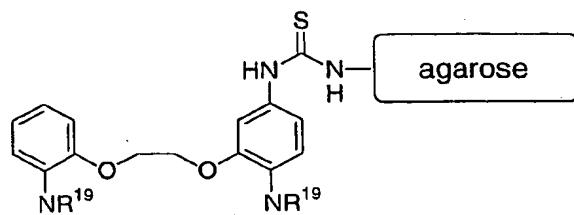
Compound 29

10 wherein R<sup>19</sup> is -(CH<sub>2</sub>CO<sub>2</sub>R<sup>13</sup>)<sub>2</sub> and R<sup>13</sup> is independently hydrogen or a salt.

70. A compound comprising a metal-chelating moiety according to Formula IV that is



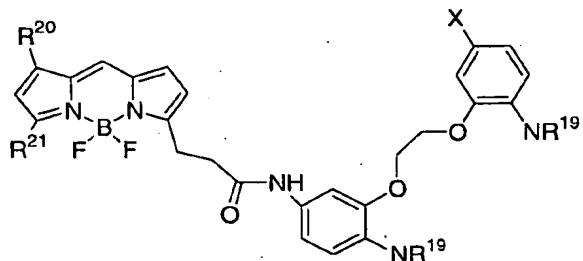
#### **Compound 14, or**



Compound 13

5 wherein R<sup>19</sup> is -(CH<sub>2</sub>CO<sub>2</sub>R<sup>13</sup>)<sub>2</sub> and R<sup>13</sup> is independently hydrogen or a salt.

71. A compound comprising a metal-chelating moiety according to Formula IV



10

wherein X is H or F; R<sup>20</sup> is H, methyl or phenyl; R<sup>21</sup> is CH<sub>3</sub>, Ph, -CH=CH-Ph, -(CH=CH)<sub>2</sub>-Ph, 2-pyrrolyl, or -CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Na;

and R<sup>19</sup> is -(CH<sub>2</sub>CO<sub>2</sub>R<sup>13</sup>)<sub>2</sub> and R<sup>13</sup> is independently hydrogen or a salt.

15

72. A ternary complex comprising a trivalent gallium ion, a phosphorylated target molecule and a phosphate-binding compound according to formula (A)m(L)n(B) wherein A is a chemical moiety, L is a linker, B is a metal chelating moiety, m is an integer from 1 to 4 and n is an integer from 0 to 4.

20

73. A ternary complex comprising a BAPTA chelating moiety, a trivalent gallium ion and a phosphorylated target molecule.

25

74. A phosphorylated target molecule determined by combining a sample comprising a phosphorylated target molecule with a binding solution according to any one of claims 1-19.